# Unusual Product Ratios Resulting from the Gamma-Irradiation of Cholesterol in Liposomes

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**Cholesterol in aqueous suspensions of multilamellar vesicles (MLV) was exposed to gamma-irradiation (0.5–10 kGy) at 0–4°C. Cholesterol oxidation products resulting from the irradiation were isolated by dry column extraction followed by preparative thin-layer chromatography (TLC) and were quantitated by on-column gas chromatography (GC). The ratio of 7-ketocholesterol/cholesterol 5,6-epoxides generated by irradiation was less than one, much lower than the ratio of ten commonly produced by autoxidation. Irradiation also produced relatively higher amounts of 7-hydroxycholesterol than did autoxidation. These unique product ratios may be suitable indicators of past exposure to irradiation.**
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As part of our search for a diagnostic test capable of discriminating meat and poultry that have been irradiated from those that have not, we have studied the effect of gamma-radiation on cholesterol in liposomes. The identification of irradiated foods has been the subject of worldwide interest (1–3), but until now no discriminating method suitable for animal tissue has been perfected. Much of the research effort in the identification of irradiated foods has been on the attempted characterization and measurement of unique radiolytic products, *i.e.* compounds whose formation can be attributed solely to the effect of ionizing radiation. The extent to which this effort has been unsuccessful, despite determined efforts of a worldwide community of talented scientists, is perhaps a measure of the trivial effect that low-dose ionizing radiation has on the chemical structure of food components.

The interaction of ionizing radiation with fatty acids, triglycerides and food fats has been studied intensively for more than twenty years, and much of this work has been summarized (4,5). Among the radiolytic products formed are hydrocarbons, aldehydes, lactones, ketones and other volatile products that are also characteristic of lipid oxidation such as would be experienced when lipids are temperature-abused in the presence of air. In model lipid systems containing polyunsaturated fatty acids, radiation-induced peroxidation has been observed and measured (6–8).

The effect of oxidizing conditions on cholesterol has been studied in detail (9), and current knowledge of cholesterol autoxidation has been reviewed recently (10,11). The principal product of the autoxidation of cholesterol in aqueous sodium stearate dispersions (12), in organic solvents (13) and in liposomes (14,15) is 7-ketocholesterol (3β-hydroxycholest-5-en-7-one). Other prominent products of cholesterol autoxidation include 7α- and 7β-hydroxycholesterol (cholest-5-ene-3β,7α-diol and cholest-5-ene-3β,7β-diol, respectively) and the α- and β-epoxide (cholesterol 5α,6α-epoxide and cholesterol 5β,6β-epoxide, respectively). In aqueous dispersions, the 7-ketone/5,6-epoxides ratio has typically been determined to range from six to ten, while about twice as much 7-ketocholesterol was formed as total 7-hydroxycholesterol (12,16).

The effect of ionizing radiation on cholesterol has also been examined in various systems, and it has been shown that the chemical changes produced are similar in nature to those known to occur during autoxidation (17–19). Recently, work performed in our laboratory revealed that irradiation of cholesterol in aqueous sodium stearate dispersions produced relatively less 7-ketocholesterol and relatively more 5,6-epoxides than did autoxidation of similar dispersions. Ratios of 7-ketocholesterol/5,6-epoxides resulting from irradiation were less than unity (20), while autoxidation, although at higher temperatures, gave ratios between six and ten (21). These results suggested that unique product ratios, rather than unique radiolytic products, might be useful indicators of past exposure to irradiation.

The current work was undertaken to determine whether cholesterol dispersed in the relatively hydrophobic medium of model membranes, *i.e.* liposomes, might also give unusual product ratios when exposed to low-dose ionizing radiation. Our earlier work had provided an instance in which ionizing radiation affected 7-ketocholesterol quite differently in liposomes than it did in aqueous dispersions (22). In that study, 7-ketocholesterol exposed to gamma radiation experienced 90% degradation in aqueous sodium stearate dispersion but only 10% degradation in liposomes.

## EXPERIMENTAL

*Materials and reagents.* Cholesterol (99 + %), 6-ketocholestanol (3β-hydroxycholestan-6-one), 7-ketocholesterol (3β-hydroxycholest-5-en-7-one), cholesterol 5α,6α-epoxide and Sephadex G 50–80 were purchased from Sigma Chemical Co. (St. Louis, MO), cholesterol 5β,6β-epoxide and 7α-hydroxycholesterol (cholest-5-ene-3β,7α-diol) from Research Plus, Inc. (Bayonne, NJ), and 7β-hydroxycholesterol (cholest-5-ene-3β7β-diol) from Steraloids, Inc. (Wilton, NH). Dipalmitoyl phosphatidylcholine (DPPC) and dipalmitoyl phosphatidylglycerol (DPPG) were obtained from Avanti Polar Lipids, Inc. (Pelham, AL). Celite 545 was purchased from Fisher Scientific (Malvern, PA), and thin-layer chromatography (TLC) plates, silica gel GHL (250 μm) from Analtech, Inc. (Newark, DE). All solvents used were "distilled in glass grade," and chemicals were of reagent grade quality. Water was double-deionized, glass-distilled.

*Liposome preparation.* Multilamellar vesicles (MLV) were prepared from 70.5 mg (96.0 μmoles) DPPC, 23.1 mg (32.0 μmoles) DPPG and 24.8 mg (64.0 μmoles) of cholesterol. The phospholipids and cholesterol were dissolved in 3.0 mL chloroform in a 15-mL test tube provided with a Teflon-lined screw cap, and the solution was mixed thoroughly. Solvent was removed under a stream of nitrogen while the tube was rotated at an angle, so that the lower half of the tube was coated evenly with a mixture of solids. The remaining solvent was removed until the odor of chloroform was no longer detectable. The coated lipids were hydrated with 8 mL water and allowed to swell for 3 hr in a water bath held at 55°C with vortexing every 15 min.

The liposome preparation was allowed to cool to room temperature and was filtered through a 0.4 μm pore polycarbonate membrane to insure rather uniform size distribution (23). The suspension was chromatographed on a 26 cm × 1 cm column of Sephadex G 50-80 using double deionized water as eluent. Vesicles were collected, as eluted, in a total volume of 16 mL. The batch was vortexed to insure homogeneity, and 1 mL aliquots under air were placed into 2-mL glass vials provided with Teflon-lined screw caps. Samples were refrigerated (0–4°C) overnight prior to irradiation. Phospholipid content of the vesicles was determined by measuring inorganic phosphorus according to the procedure of Eng and Noble (24).

*Irradiation of cholesterol in liposomes.* Duplicate 1-mL aliquots of MLV were irradiated in a $^{137}Cs$ source (0.118 KGy/min) at 0–4°C at dose levels of 0.5, 1.0, 2.5, 5.0, or 10.0 KGy. Controls consisted of unirradiated, refrigerated (0–4°C) samples. Controls and irradiated samples were extracted immediately after irradiation.

*Autoxidation of cholesterol in liposomes.* Liposome suspension (10 mL) was placed in a 50-mL screw cap test tube equipped with magnetic stirring bar. The tube was sealed with air in the headspace. The tube was suspended in a water bath kept at 45°C and stirred for 48 hr. Unoxidized aliquots were kept 0–4°C. Duplicate 1-mL portions of oxidized and unoxidized controls were analyzed.

*Dry column extraction of cholesterol oxides.* Anhydrous, granular sodium sulfate (4.0 g) and Celite 545 (3.0 g, previously washed with methylene chloride) were ground in a porcelain mortar, and 1 mL of a liposome preparation was applied to the powder evenly. The mixture was ground thoroughly for three minutes and transferred in small increments to a 10-mL glass syringe containing a thin pad of glass wool that had been previously washed with methylene chloride. The dry mixture was tamped firmly into the syringe with a flattened glass rod to a total volume of 6–7 mL. The mortar was rinsed with 5 mL methylene chloride, and the rinse was added to the solids in the syringe. Additional methylene chloride was added gently to the top of the column until 10 mL eluate had been collected at a flow rate of <1 mL/min. Eluates were blanketed with nitrogen and stored in the refrigerator (0–4°C) pending isolation and analysis.

*Isolation of cholesterol oxidation products.* Each dry column eluate was evaporated to dryness with nitrogen. The residue was reconstituted in 250 μL chloroform and the solution streaked on the middle 10 cm section of a scored 20 × 20 cm TLC plate. The plate had been prewashed with chloroform/methanol (2:1,v/v), again with ethyl acetate, and activated at 110°C. The residue container was rinsed with 150 μL chloroform and the rinse streaked over the previously applied sample. The end sections of the scored plate were spotted with 2 μL each of 7-ketocholesterol and 7α-hydroxycholesterol solution (1 mg/mL). The plate was developed with benzene/ethyl acetate (60:40,v/v) and development continued for 10 min after the solvent front had reached the top of the plate. The end sections, intended for visualization, were snapped off and sprayed with 50% sulfuric acid and heated. They were used to locate the band containing both 7-ketocholesterol and 5,6-epoxide and the 7-hydroxycholesterol band in the center section of the plate. The corresponding bands of the middle section were scraped and separately extracted with 2 × 5 mL acetone. After each extraction the samples were centrifuged to pellet the silica. The two extracts of each band were combined, filtered through a 2 μm Nylon 66 filter and evaporated to dryness under nitrogen. To each residue was added internal standard 6-ketocholestanol (4 μL, 1 μg/μL in ethyl acetate), and the solvent was removed with nitrogen. The sample was reconstituted in 100 μL ethyl acetate for gas chromatography (GC) analysis.

*Determination of cholesterol content of liposomes.* The sample of MLV (1 mL) was ground with anhydrous sodium sulfate (4.0 g) and Celite 545 (3.0 g) as described above. Before the ground mixture was transferred to the 10-mL syringe, the latter was provided with 1.0 g of a mixture of Celite 545/$CaHPO_4 \cdot 2H_2O$ (9:1, w/w) which was tamped securely. Addition of the dry powder containing the sample then brought the total volume in the syringe to 8–9 mL. The column was eluted with methylene chloride, and approximately 10 mL of eluate was collected. Solvent was removed under nitrogen and the sample was reconstituted in methylene chloride to 10.0 mL in a volumetric flask. An aliquot (10 μL) was transferred to a vial, internal standard 6-ketocholestanol (4 μL, 1 μg/μL in ethyl acetate) was added, the solvent was removed under nitrogen, and the residue was reconstituted in 100 μL ethyl acetate prior to GC analysis.

*Gas chromatography (GC).* Samples were analyzed by GC as described previously (25) with the following modifications. The column was a 0.25 mm i.d. × 30 m bonded phase 5% phenylsilicone column with 0.25 μm film thickness (Durabond 5, J & W Scientific, Folsom, CA). A retention gap was not used. For cholesterol oxide determinations, the initial column temperature of 100°C was held 2 min, then programmed at 30°C/min to 265°C, then at 0.5°C/min to 275°C where it was held for 5 min, then programmed at 5°C/min to 300°C and held for 15 min. For cholesterol determinations the program was modified by programming from 275 to 300°C at 30°C/min and holding at 300°C for only 2 min. Sample size injected in all cases was 0.6–0.8 μL.

*GS response factors.* Response factors of cholesterol and of the five cholesterol oxidation products of interest relative to the internal standard 6-ketocholestanol were determined by the method of Lee *et al.* (26).

## RESULTS AND DISCUSSION

Various types of liposomes have been employed as model membrane systems to study the reactions of lipids, including their peroxidation (27), in environments that approach those of biological membranes. We chose to study the effect of ionizing radiation in multilamellar vesicles (MLV), because these types of liposomes are easily prepared in the quantities that we required. In the current series of experiments the MLV were composed of the fully saturated phospholipids dipalmitoyl phosphatidylcholine (DPPC) and dipalmitoyl phosphatidylglycerol (DPPG), and cholesterol. The molar ratio DPPC/DPPG/cholesterol was 3:1:2. The liposomes were formed in double deionized water, because exploratory experiments with *N*-2-hydroxyethyl piperazine-*N'*-2-ethane sulfonic acid (HEPES) buffer and with phosphate buffer had indicated objectionable interactions of the buffer with the radiation. The liposomes were formed and swelled above 41°C, the transition temperature ($T_c$) for both phospholipids (28).

Swelling of the proper lipid components in aqueous media above the $T_c$ of the lipids leads to spontaneous vesiculation with the formation of liposomes (29). While liposome formation is spontaneous, it is not necessarily complete. One of the techniques used to free liposomes from non-trapped solutes is molecular sieve chromatography (27,30). Table 1 lists six experiments in which liposomes were prepared under supposedly identical conditions, including separation of unincorporated lipids on a Sephadex G 50-80 column. The data indicate that 66–77% of the added cholesterol was found incorporated in the liposomes, while 80–96% of the phospholipids added were present in the vesicles. The actual cholesterol content of the liposomes ranged from 28–32 mol%, slightly different than the intended 33 mol%. The MLV were characterized by analysis of total phosphorus content (24) and by cholesterol determination.

To determine cholesterol and its major oxides it is necessary to extract the compounds from the vesicles. Since the sterols are fairly soluble in ethyl acetate while the phospholipids are not, a simple extraction was thought to be necessary. However, repeated extractions of the liposomes with ethyl acetate revealed that cholesterol was retained by the liposomes tenaciously, so that after five consecutive extractions more than 5% of the cholesterol remained in the vesicles. Jacobsohn *et al.* (31,32) also reported retention of cholesterol in MLV containing phosphatidylcholine and suggested that the phospholipid in the aqueous phase prevents access to cholesterol by nonpolar solvents. The difficulty of quantitative extraction of sterols from foods has also been noted (33).

Extraction of cholesterol from phospholipids should be facilitated if the aqueous phase is removed first. The dry column extraction procedure (34) is designed to remove water from partly aqueous systems such as animal tissue, and seemed suitable for the dehydration of liposomes. For the purposes of the current work the procedure was modified, so that a 10-mL glass syringe

**TABLE 1**

**Cholesterol and Phosphorus Content of Liposomes**

| | Cholesterol | | | | Phosphorus ($P_i$) | | |
|---|---|---|---|---|---|---|---|
| Experiment | Amount added μmol/mL | Amount found μmol/mL | Percent incorporated in liposomes | Liposome content mol% | Amount added μmol/mL | Amount found μmol/mL | Percent incorporated in liposomes |
| 1 | 4.01 | 2.64 | 65.8 | 28.9 | 8.00 | 6.51 | 81.4 |
| 2 | 4.01 | 3.04 | 75.8 | 29.7 | 8.00 | 7.20 | 90.0 |
| 3 | 4.01 | 2.90 | 72.3 | 28.7 | 8.00 | 7.20 | 90.0 |
| 4 | 4.01 | 2.94 | 73.3 | 27.8 | 8.00 | 7.64 | 95.5 |
| 5 | 4.01 | 3.09 | 77.1 | 31.0 | 8.00 | 6.88 | 86.0 |
| 6 | 4.01 | 3.03 | 75.5 | 31.9 | 8.00 | 6.47 | 80.5 |

**TABLE 2**

**Purities and GC Response Factors of Various Cholesterol Oxidation Products**

| Compound | Purity | Response factor[a] |
|---|---|---|
| Cholesterol (5-cholesten-3β-ol) | 95.1 | 0.87 |
| α-Epoxide (cholesterol 5α,6α-epoxide) | 100.0 | 1.10 |
| β-Epoxide (cholesterol 5β,6β-epoxide) | 96.0 | 1.09 |
| 7-Ketocholesterol (3β-hydroxycholest-5-en-7-one) | 96.8 | 1.03 |
| 6-Ketocholestanol (3β-hydroxycholestan-6-one) | 94.8 | 1.00 |
| 7α-Hydroxycholesterol (cholest-5-ene-3β,7α-diol) | 93.2 | 1.11 |
| 7β-Hydroxycholesterol (cholest-5-ene-3β,7β-diol) | 97.1 | 1.11 |

[a]Relative to 6-ketocholestanol.

could serve as the extraction column. Since cholesterol was the only neutral lipid in the liposomes, methylene chloride was a suitable solvent for its extraction. Elution with 10 mL of this solvent gave complete recovery of cholesterol, as judged by TLC. Unfortunately, however, small but significant amounts of phospholipids co-eluted. The latter compounds are not volatile in our GC procedure and constituted a potential contaminant for our capillary column.

Three procedures were investigated to overcome this difficulty. Reduction of the methylene chloride eluate in volume followed by preparative TLC gave a discrete cholesterol band, well resolved from the phospholipid band which remained at the origin. Extraction of the scraped cholesterol band with acetone led to a clean GC trace of cholesterol suitable for quantitation. This procedure was rather tedious and sometimes gave erratic recoveries. In an alternative procedure, 1-mL aliquots of liposome suspensions were saponified in a modification of the procedure of Slover *et al.* (35), which was effective in destroying phospholipids and hence made extraction of cholesterol feasible. In our hands this technique lacked reproducibility and was time-consuming, and hence was abandoned. Finally, the dry column procedure was modified by insertion of a Celite/$CaHPO_4$ trap (see Experimental) to minimize elution of the phospholipids. The technique gave 86% recoveries when applied to liposome suspensions containing known amounts of cholesterol, *i.e.* vesicles that were allowed to swell but were not filtered or passed through molecular sieves. The cholesterol values listed in Table 1 were determined by this method but were not adjusted to compensate for the 14% loss mentioned above.

Recovery and measurement of cholesterol oxidation products from irradiated liposomes was also carried out with use of the dry column procedure (no trap) followed by preparative TLC. To test the procedure, liposomes were prepared that, in addition to the usual components, also contained known amounts of 7-ketocholesterol (9.7 μg/mL of suspension), the two isomeric 5,6-epoxides (31.6 μg/mL) and the two isomeric 7-hydroxycholesterols (31.3 μg/mL). The vesicles were swelled but not filtered or passed through the Sephadex column to avoid losses of compounds that had failed to be incorporated into the MLV. Recovery of 7-ketocholesterol (92.9%) and 7-hydroxycholesterols (91.8%) validated the procedure. The two 5,6-epoxides, however, were unstable to the vesicle swelling conditions (55°C, pH 6.4) and were largely converted to 5α-cholestane-3β,5,6β-triol, as expected from a previous study (36). The triol was incompletely resolved from the phospholipids by TLC and was recovered only partially. Total recovery of 5,6-epoxides and triol was 63.4%.

Purities of standard compounds as determined by GC and GC response factors relative to the internal standard are shown in Table 2. The response factors were used in calculating the data reported in Table 3. The low response factor of cholesterol may be a function of its lower-than-expected purity.

Table 3 lists the ratios of 7-ketocholesterol/5,6-epoxides generated as a result of exposure of cholesterol in MLV to ionizing radiation at 0–4°C. The ratios of all irradiated samples are less than unity, and actu-

TABLE 3

Cholesterol Oxidation Products Resulting from γ-Irradiation of Cholesterol in Liposomes[a,b]

| Dose kGy | μg/mL | | | | | | | Ratio | Ratio |
|---|---|---|---|---|---|---|---|---|---|
| | 7-keto-cholesterol | Cholesterol 5β,6β-epoxide | Cholesterol 5α,6α-epoxide | 7α-hydroxy-cholesterol | 7β-hydroxy-cholesterol | Total oxides measured | 7-ketone/ 5,6-epoxides | 7-ketone/ 7-hydroxy-cholesterols |
| 0 | 0.50 ± 0.27 | 0.36 ± 0.12 | 0.20 ± 0.11 | trace | trace | 1.49 ± 0.75 | 0.86 ± 0.11 | — |
| 0.5 | 0.65 ± 0.15 | 3.10 ± 1.21 | 0.75 ± 0.24 | 0.38 ± 0.06 | 0.38 ± 0.11 | 5.26 ± 1.52 | 0.20 ± 0.12 | 0.85 ± 0.10 |
| 1.0 | 1.46 ± 0.48 | 5.60 ± 2.42 | 1.30 ± 0.46 | 0.90 ± 0.34 | 0.99 ± 0.44 | 10.25 ± 3.90 | 0.22 ± 0.05 | 0.79 ± 0.11 |
| 2.5 | 1.60 ± 0.26 | 7.46 ± 2.48 | 1.65 ± 0.50 | 5.30 ± 1.44 | 3.84 ± 1.23 | 19.84 ± 5.49 | 0.19 ± 0.05 | 0.19 ± 0.04 |
| 5.0 | 1.82 ± 0.50 | 8.96 ± 4.27 | 1.95 ± 0.77 | 6.07 ± 2.02 | 3.89 ± 1.48 | 22.65 ± 9.07 | 0.18 ± 0.05 | 0.19 ± 0.02 |
| 10.0 | 2.89 ± 0.93 | 10.1 ± 2.41 | 2.32 ± 0.44 | 8.01 ± 2.61 | 4.52 ± 1.05 | 27.84 ± 5.80 | 0.24 ± 0.07 | 0.24 ± 0.01 |

[a] Amounts corrected for GC response factors.
[b] Mean value of 6 samples ± standard deviation.

ally <0.35 in most experiments. The reason for the slight increase in the ratio at higher doses in some experiments requires further investigation. The unirradiated vesicles contained a somewhat higher ratio of 7-ketone/5,6-epoxide as would be expected, since these oxidation products were formed during the liposome swelling process in the presence of air, rather than by means of ionizing radiation. In addition it should be noted that the levels of 7-ketone and 5,6-epoxides in the unirradiated samples are very low, so that a greater amount of uncertainty is involved in their quantification. Irradiation, even at the low dose of 0.5 kGy, increases the amounts of cholesterol oxidation products greatly, so that these compounds can be measured at this dose with good precision.

The ratios of 7-ketone/5,6-epoxides produced by exposure of cholesterol to γ-radiation in the presence of air can best be understood by comparing them to the ratios of the same products, *i.e.* 6–10 (see introduction) generated by the principal alternative free radical reaction, autoxidation. It is apparent that the ratios that are now seen to result from irradiation, *i.e.* <1.0, are quite different from those generated by autoxidation. Hence in the current work concerning cholesterol in saturated liposomes the signature of the irradiation process is unique and easily distinguishable from the principal competing process, autoxidation.

In order to determine whether the higher 7-ketone/5,6-epoxides ratio holds for the autoxidation of cholesterol in saturated liposomes, a suspension of such vesicles was exposed to air oxidation at 45°C for 48 hr and the products were isolated. About 20% of the cholesterol oxidized during that period and the experimental 7-ketone/5,6-epoxide ratio was 10.6 ± 3.0.

It has been reported (37) that γ-irradiation of dispersions of cholesterol gave rise to a distinctly different ratio of 5α,6α-epoxide 5β,6β-epoxide, *i.e.* 3.5, than that obtained by autoxidation (0.1). If confirmed, the α/β-epoxide ratio might also be a useful marker to distinguish irradiated from autoxidized cholesterol. In our earlier study of the irradiation of cholesterol in aqueous dispersions, we reported α/β-epoxide ratios of less than one, usually 0.3–0.4. In the current work the irradiation of cholesterol in liposomes gave an α/β-epoxide ratio of 0.2–0.3 consistently. It must be concluded that this ratio cannot be used as evidence for past irradiation.

Table 3 contains a list of the ratios of 7-ketocholesterol/7-hydroxycholesterols obtained when cholesterol in MLV was irradiated at 0–4°C with 0.5 to 10.0 kGy of γ-radiation. With the exception of two values at 0.5 kGy, all of the ratios are less than unity and most of them are less than 0.5. In calculating the values of Table 3, the 7α- and 7β-hydroxycholesterols, determined individually, but not always resolved completely, have been added.

Again it is useful to compare the ratios of Table 3 with those reported to have been produced by autoxidation. In aqueous dispersions, autoxidation of cholesterol produced about twice as much 7-ketocholesterol as 7-hydroxycholesterol (11,12,16,21), while uncatalyzed autoxidation of cholesterol in liposomes has been reported to give a ratio of about 1.5 (19). In the current work, autoxidation of cholesterol in liposomes gave a 7-ketone/7-hydroxycholesterol ratio of 3.4 ± 0.6. It is apparent, then, that the difference in this ratio between irradiated and autoxidized cholesterol is not as clear-cut as it is for the 7-ketocholesterol/5,6-epoxide ratio. Nevertheless, the two ratios taken together may serve as strong evidence of past irradiation.

Finally, there is the matter of the total amounts of measured cholesterol oxidation products. These include 7-ketocholesterol, the isomeric 5,6-epoxides, and the isomeric 7-hydroxycholesterols. Their sums are shown in Table 3. The level of oxidation products in the unirradiated samples is quite low. In a previous study (20), an aqueous dispersion of cholesterol was subjected to a flow of air at 0–4°C for two weeks without a detectable increase in cholesterol oxidation.

The amounts of cholesterol oxidation products increase dramatically with radiation dose, but the increase does not appear to be linear. As can be seen from Table 3, we experienced some difficulties in experiment-to-experiment reproducibility of the amounts, but not of the ratios, of cholesterol oxides. This in spite of exhaustive, detailed studies of the variables of each step of the procedure. Our investigations along these lines are continuing.

This research has clearly demonstrated that γ-irradiation of cholesterol, contained in MLV prepared from saturated phospholipids, leads to ratios of oxidation products that are substantially different from those generated by autoxidation. New procedures for isolating and measuring cholesterol and its oxidation products in aqueous liposome suspensions have been developed in the course of these studies and are reported here. Further research is in progress to identify and to define reaction parameters.

## REFERENCES


1. Delincée, H., and Ehlerman, D.A.E. (1989) *Radiat. Phys. Chem., Int. J. Radiat. Appl. Instrum., Part C 34*, 877–890.
2. Grootveld, M., and Jain, R. (1989) *Radiat. Phys. Chem., Int. J. Radiat. Appl. Instrum., Part C 34*, 925–934.
3. Heide, L., Guggenberger, R., and Bögl, K.W. (1989) *Radiat. Phys. Chem., Int. J. Radiat. Appl. Instrum., Part C 34*, 903–913.
4. Nawar, W.W. (1977) in *Radiochemistry of Major Food Components* (Elias, P.S., and Cohen, A.J., eds.) pp. 21–61, Elsevier Scientific Publishing Co., New York.
5. Nawar, W.W. (1983) in *Preservation of Food by Ionizing Radiation* (Josephson, E.S., and Peterson, M.S., eds.) Vol. 2, pp. 75–125, CRC Press, Inc., Boca Raton.
6. Hasegawa, K., and Patterson, L.K. (1978) *Photochem. Photobiol. 28*, 817–823.
7. Nakazawa, T., and Nagatsuka, S. (1980) *Int. J. Radiat. Biol. 38*, 537–544.
8. Nagatsuka, S., and Nakazawa, T. (1982) *Biochim. Biophys. Acta 691*, 171–177.
9. Smith, L.L. (1981) *Cholesterol Autoxidation*, Plenum Press, New York.
10. Smith, L.L. (1987) *Chem. Phys. Lipids 44*, 87–125.
11. Maerker, G. (1987) *J. Am. Oil Chem. Soc. 64*, 388–392.
12. Kimura, M., Jin, Y., and Sawaya, T. (1979) *Chem. Pharm. Bull. (Tokyo) 27*, 710–714.
13. Muto, T., Tanaka, J., Miura, T., and Kimura, M. (1982) *Chem. Pharm. Bull. (Tokyo) 30*, 3172–3177.
14. Terao, J., Sugino, K., and Matsushita, S. (1985) *J. Nutr. Sci. Vitaminol. 31*, 499–508.
15. Lijana, R.C., McCracken, M.S., and Rudolph, C.J. (1986) *Biochim. Biophys. Acta 879*, 247–252.

16. Bergström, S., and Wintersteiner, O. (1942) *J. Biol. Chem. 145*, 309-326.
17. Ansari, G.A.S., and Smith, L.L. (1979) *Photochem. Photobiol. 30*, 147-150.
18. Kucera, J., Schwarz, V., and Sykora, M. (1984) *J. Radioanal. Nucl. Chem. 87*, 219-230.
19. Sevanian, A., and McLeod, L.L. (1987) *Lipids 22*, 627-636.
20. Lakritz, L., and Maerker, G. (1989) *J. Food Sci. 54*, 1569-1572.
21. Zulak, I.M., and Maerker, G. (1989) *J. Am. Oil Chem. Soc. 66*, 1499-1503.
22. Moriarity, N.J., and Maerker, G. (19xx) *J. Am. Oil Chem. Soc. 67*, 923-926.
23. Olson, F., Hurt, C.A., Szoka, F.C., Vail, W.J., and Papahadjopoulos, D. (1979) *Biochim. Biophys. Acta 557*, 9-23.
24. Eng, L.F., and Noble, E.P. (1968) *Lipids 3*, 157-162.
25. Maerker, G., and Unruh, J., Jr. (1986) *J. Am. Oil Chem. Soc. 63*, 767-771.
26. Lee, M.L., Yang, F.J., and Bartle, K.D. (1984) *Open Tubular Column Gas Chromatography. Theory and Practice*, p. 222. John Wiley & Sons, New York.
27. Chatterjee, S.N., and Agarwal, S. (1988) *Free Radical Biol. Med. 4*, 51-72.
28. Szoka, F.C., and Papahadjopoulos, D. (1980) *Ann. Rev. Biophys. Bioeng. 9*, 467-508.
29. Madden, T.D., Tilcock, C.P.S., Wong, K., and Cullis, P.R. (1988) *Biochemistry 27*, 8724-8730.
30. Chapman, D. (1984) in *Liposome Technology, Vol. I Preparation of Liposomes* (Gregoriadis, G., ed.) pp. 1-8, CRC Press, Inc., Boca Raton.
31. Jacobsohn, M.K., Esfahani, M., and Jacobsohn, G.M. (1986) *Lipids 21*, 691-696.
32. Jacobsohn, M.K., Bazillian, L.S., Hardiman, J., and Jacobsohn, G.M. (1989) *Lipids 24*, 375-382.
33. Lognay, G., Dreze, P., Wagstaffe, P.J., Marlier, M., and Severin, M. (1989) *Analyst 114*, 1287-1290.
34. Maxwell, R.J., Marmer, W.N., Zubillaga, M.P., and Dalickas, G. (1980) *J. Assoc. Off. Anal. Chem. 63*, 600-603.
35. Slover, H.T., Thompson, R.H., and Merola, G.V. (1983) *J. Am. Oil Chem. Soc. 60*, 1524-1528.
36. Maerker, G., and Bunick, F.J. (1986) *J. Am. Oil Chem. Soc. 63*, 771-777.
37. Gumulka, J., St. Pyrek, J., and Smith, L.L. (1982) *Lipids 17*, 197-203.